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FORMATION OF ADRENALINE FROM *NOR*ADRENALINE IN THE PERFUSED SUPRARENAL GLAND

BY

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Observations on the secretion of adrenaline from the perfused suprarenal gland of the dog have been described by Bülbring, Burn, and de Elío (1948). These experiments were carried out before the publication of work by various authors, Schümann (1948), Bülbring and Burn (1949a and b) and v. Euler and Hamberg (1949), showing that the suprarenal medulla contains *nor*adrenaline as well as adrenaline, and no tests were used which would have distinguished between the two substances. The numerous estimations were made with isolated loops of rabbit intestine, comparing the samples with adrenaline only. The main outcome was to show that the liberation of adrenaline-like material in the blood leaving the gland was modified by the presence of adrenaline in the perfusing blood. When this was very small in amount, stimulation of the splanchnic nerve liberated very little adrenaline-like material in the venous blood. When the experiment was performed with progressively larger amounts of adrenaline in the perfusing blood, stimulation of the splanchnic nerve liberated progressively larger amounts of adrenaline-like material until an optimum concentration for adrenaline in the perfusing blood was reached. A further increase of adrenaline in the perfusing blood beyond this optimum produced a steady decline in the amount of adrenaline-like substance liberated by splanchnic stimulation.

The work had, however, been begun with the object of studying the formation of adrenaline by the isolated gland, and in the experiments in which less than 1 μg. adrenaline per ml. was present in the perfusing blood, an increase in the amount of adrenaline-like material in the system was always observed. Meanwhile West (1947), working in the pharmacological laboratory in Edinburgh, had shown that the rat uterus was much more sensitive to adrenaline than to *nor*adrenaline. When used as described by de Jalon, Bayo, and de Jalon (1945) this test can be used to estimate adrenaline in a mixture of the two drugs (see Gaddum, Peart, and Vogt, 1949).

We therefore carried out a series of five perfusion experiments in which we estimated adrenaline and *nor*adrenaline separately throughout each experiment, and we now give a brief description of the results which were obtained.

METHODS

The experiments were performed as already described by Bülbring, Burn, and de Elío (1948), the right gland being first removed in order to determine the content of adrenaline and *nor*adrenaline per g.; this was assumed to be the content of the left gland at the beginning of the perfusion. All venous blood from the perfused gland was collected in a series of samples, and after the cells had been spun off, the plasma was tested. The left gland was tested at the end of the perfusion. By comparisons with adrenaline on the rabbit intestine a figure was obtained which we took as the sum of adrenaline + *nor*adrenaline. By comparisons with adrenaline on the rat uterus we obtained a figure which we took as the amount of adrenaline only.

RESULTS

Perfusion was carried out for periods which varied in different experiments from 2 hr. 7 min. to 3 hr. 25 min. The rate of flow through the gland diminished during the course of the perfusion. The following are the details of an experiment in which the flow was good throughout, starting at the rate of 12 c.c./min. and being 2.25 c.c./min. at the termination 2 hr. 19 min. later.

Experiment No. 1.

Right gland	Total	2,391 μg. or	1.65 mg./g.
	Adrenaline	1,162 μg	0.8 mg./g.
(by difference)	*Nor*adrenaline	1,229 μg.	0.85 mg./g.

The left gland weighed 1.6 g. Its active content at the beginning of the perfusion was assumed to be the same per g. as that of the right gland; hence

Left gland	Total	2,620 μg.
	Adrenaline	1,260 μg.
	*Nor*adrenaline	1,360 μg.

At the end of the perfusion the contents of the left gland were found to be

Total	2,062 μg.
Adrenaline	1,762 μg.
*Nor*adrenaline	300 μg.

During the perfusion the splanchnic nerve was stimulated continuously for 1 hr., and at the end of this period *nor*adrenaline was added to the perfusing blood so that the concentration present in terms of *l-nor*adrenaline was 5.4 μg./c.c. During the rest of the perfusion 144 c.c. of blood passed through the gland so that the amount of *nor*adrenaline added to the system in this way was 777 μg. In the venous blood from the perfused gland there was found

Total	1,073 μg.
Adrenaline	307 μg.
*Nor*adrenaline	766 μg.

The following balance could therefore be drawn

	At beginning	At end
Adrenaline in gland ..	1,260 μg.	1,762 μg.
,, in venous blood		307 μg.
*Nor*adrenaline in gland ..	1,360 μg.	300 μg.
,, added to blood ..	777 μg.	
,, in venous blood ..		766 μg.
	3,397 μg.	3,135 μg.

The figures for the totals at the beginning and at the end show fairly close agreement, there being a loss of less than 8 per cent. There was, however, a large loss of 1,071 μg. *nor*adrenaline and a large gain of 809 μg. adrenaline. The evidence supports the view that during the perfusion *nor*adrenaline was converted to adrenaline.

Of the four other experiments one was unsatisfactory because of the poor rate of flow through the gland. There was little change in the total contents of the system though the stimulation of the splanchnic nerve liberated both adrenaline and some *nor*adrenaline in the venous blood. The balance was as follows:

Experiment No. 2.

	At beginning	At end
Adrenaline in gland ..	1,215 μg.	415 μg.
,, in venous blood		821 μg.
*Nor*adrenaline in gland ..	0 μg.	85 μg.
,, added to blood ..	155 μg.	
,, in venous blood		244 μg.
	1,370 μg.	1,565 μg.

The total adrenaline was almost identical in amount at the end as at the beginning (1,236 μg. as compared with 1,215 μg.) though two-thirds of it appeared in the venous blood. The total *nor*adrenaline rose from 155 μg. to 329 μg. This change was an increase of 174 μg. on an initial total of 1,370 μg., that is to say, it was 12.7 per cent. The three remaining experiments were satisfactory. Since the results were similar to those in Exp. 1 they have been summarized together in Table I.

TABLE I

CHANGES OBSERVED DURING PERFUSIONS

Exp.	Total		Adrenaline		*Nor*adrenaline	
	μg.	%	μg.	%	μg.	%
1	−262	−8	+809	+64	- 1,071	−51
3	+235	+22	+315	+40	- 80	−26
4	+226	+16	+292	+29	- 66	−17
5	−503	−16	+315	+18	- 810	−61

The first point shown in the Table is that in each experiment there was a gain in adrenaline, and in each there was a loss in *nor*adrenaline. Even in the two experiments in which there was a net loss, there was nevertheless a gain in adrenaline, and in those in which there was a net gain this was exceeded by the gain in adrenaline. The changes have also been expressed as percentages of the amounts originally present. These percentages are large enough to show that the absolute changes are not so small as to be within the error of the biological methods used.

When each experiment is taken singly there is some correspondence between the loss of *nor*adrenaline and the gain in adrenaline in experiments Nos. 1 and 5, but not in experiments Nos. 3 and 4, in which the recorded loss of *nor*adrenaline was small. In experiments 3 and 4 the glands contained adrenaline only, both at the beginning and at the end of perfusion, while in experiments 1 and 5 the change shown in Table II took place. In these

TABLE II

CONTENTS OF GLAND

	At beginning		At end	
No. of exp.	% Adr.	% *Nor.*	% Adr.	% *Nor.*
1	48	52	86	14
5	73	27	96	4

experiments both glands contained initially a considerable amount of *nor*adrenaline which at the end of the perfusion was very small. This finding also suggests that *nor*adrenaline was converted into adrenaline.

Discussion

The experiments furnish evidence that when the dog's suprarenal gland is isolated from all other tissues and is perfused with heparinized blood from a pump, *nor*adrenaline is methylated to form adrenaline in the course of the perfusion. A change consistent with this conversion was observed in each of four satisfactory experiments. In a fifth experiment in which the blood flow was poor there was no evidence of this change. The absence of conversion in this experiment added weight to the evidence of conversion in the others.

In each experiment the right suprarenal was analysed, without having been perfused, as a control, and in three of the five glands so examined *nor*adrenaline was found present as well as adrenaline. In these the *nor*adrenaline constituted respectively 14, 28, and 52 per cent of the total. In two of the glands, however, we found only adrenaline, and in glands taken from two other dogs also we found only adrenaline. For this reason *nor*adrenaline was added to the perfusing blood in the perfusion experiments.

These results agree with the evidence of Bülbring (1949) that minced suprarenal tissue transforms *nor*adrenaline to adrenaline. She observed that this process of methylation was much more active when the glands had first been depleted by a period of splanchnic stimulation. The splanchnic nerve was stimulated in experiments 1 and 3 of the perfusions, and the percentage increase in adrenaline was greater in these experiments than in the others in which the splanchnic nerve was not stimulated.

Summary

1. The dog's suprarenal gland may contain not only adrenaline, but also *nor*adrenaline. Out of seven glands three were found to contain both these substances.

2. When the gland was perfused with heparinized blood, *nor*adrenaline being added to this blood, an increase in the amount of adrenaline was observed coinciding with a decrease in the amount of *nor*adrenaline. The conversion of *nor*adrenaline to adrenaline is indicated.

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